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6517 Montrose Avenue
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June 7, 1994

Box DD
Commissioner of Patents and Trademarks
Washington, D.C. 20231

Dear Sir or Madam:

This letter discloses additional data for my invention, patent applied for August, 1993, serial #08/104,529. I am including more data to support my claims. The current disclosure pertains to data on MRL/lpr mice. A study was performed to show that methods of immunization that prevented diabetes in NOD mice and BB rats would also prevent spontaneous autoimmunity in MRL mice which develop a disease very similar to Lupus in humans. The mice, as with human Lupus patients develop a fatal immune complex glomerunephritis if they do not receive medical care. The data below proves that the methods used in the patent application can prevent multiple autoimmune diseases.

Pregnant MRL/MpJ-lpr mice were ordered from Jackson Laboratory (Bar Harbor, Maine) on 1/12/94, and shipped 2/15/94. The pups born to these mice were injected with PBS or a combination of the anthrax vaccine and the acellular DTP vaccine. A series of nine intraperitoneal injections of the vaccines diluted in PBS were given to newborn mice using the following protocol: day 1 (.1 ml, 1:100), day 3 (.1 ml, 1:100), day 10 (.15 ml, 1:100 ml), week 4 and every 2 weeks through week 14 (.2 ml, 1:50). Vaccines were mixed prior to injection so volume in the notation refers to total volume injected. Weaning was done at approximately 21 days and only the female mice were saved.

Urine was screened for the presence of protein, a common clinical test for glomerulonephritis. A few drops of mouse urine were placed on a urine protein dipstick (Albustix, Miles Inc, Elkhart, In). At 13 weeks 6/38 (16%)of PBS control mice had a urine with a protein over 300mg/dl while 0/28 of the vaccinated mice had a urine with a protein over 300mg/dl. At 14 weeks 10/38 (26.3%) of PBS treated control mice had a urine with protein of over 200mg/dl while 0/26 of the vaccinated mice had developed urines with similar protein levels. Most (8/10) of those with positive urines had protein levels at or above 300mg/dl. All those with high urine proteins at 13 weeks of life continued to have high urine proteins on week 14 verifying the reliability of the assay. The results prove that the vaccination methods in the patent application prevent multiple autoimmune diseases.

Sincerely,

John B. Classen, M.D., M.B.A.